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UNITED STATES DEPARTMENT OF AGRICULTURE

BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE

FOREST INSECT INVESTIGATIONS

MEMORANDUM FOR ME. MILLER

RE: RELATION OF FAT AND MOISTURE CONTENT TO COLD HARDINESS IN LARVAE OF D. MONTICOLAE AND D. BREVICOMIS

by
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Berkeley, California
March 31, 1938

U. S. GOVERNMENT PRINTING OFFICE 60918

MEMORANDUM FOR MR. MILLER.

RE: RELATION OF FAT AND MOISTURE CONTENT TO COLD HARDINESS IN LARVAE OF D. MONTICOLAE AND D. BREVICOMIS.

This memorandum covers the results obtained by the new extraction method; for the sake of continuity and comment, the material contained in the memorandum of December 31, 1937 has been repeated here.

METHODS

A. Fat extraction and moisture determination.

As mentioned before, the extraction with di-ethyl ether was abandoned because of oxidation of the lipids. The present method has been evolved with the help of biochemists of the University, one of whom is a recognized authority on the chemistry of fats. An outline of the procedure is as follows:

1. The size of sample has varied from 50 to 150 larvae depending upon the numbers available; satisfactory results have not been obtained with less than 50 and larger numbers are desirable.

2. For determination of moisture content, a portion of the sample, 10 or more larvae, is selected by random choice. The larvae are weighed, killed by quickly dipping in hot water (60° C), dried 24 hours at 50° C and the dry weight then determined. The drying has been done at 50°C because the higher the temperature the greater the possibility of removing water held in chemical combination or of changing other body constituents and this is approximately the lowest temperature at which the larvae will approach a constant weight within 24 hours. For greatest accuracy, tissue should be dried in a vacuum oven, but such equipment is not available.

3. The remaining larvae of the sample are weighed and killed as above and then ground with sand in a glass extraction thimble. The sand used has previously been freed of soluble salts and organic matter. The grinding is done by means of a small glass pestle driven by an electric motor.

4. The thimble containing the ground larvae is placed in a modified Soxhlet apparatus; extracted 4 hours with hot alcohol, and re-extracted for one hour with petroleum ether (redistilled 30° to 50° C B. pt.).

5. The two extracts are now combined in a washer; distilled water added and washed with additional petroleum ether for 1½ hrs. The extracted lipid is then freed from the petroleum ether by drawing the latter off by low vacuum and in an atmosphere of nitrogen to prevent oxidation. The free lipid is then weighed and stored at -7° C.

B. Saponification number.

The standard method has proven unsatisfactory for the small amounts used in this work. Dr. Craig of the Division of Entomology at the University has been working on a method which requires only 50 to 100 milligrams of lipid. The procedure is essentially as follows:

1. Weigh out 50 to 100 milligrams of fat.

2. Boil ½ hour with alcoholic KOH.

3. Acidify with HCl.

4. Back titrate with 0.1 N KOH plotting the titration curve from p H 3.5 to 8.5.

5. Calculate from curve the milligrams of KOH required to saponify 1 gram of lipid.

This method is still in the developmental stage and while consistent results are obtained with fats of similar composition, the comparison of fats of widely different composition may lead to considerable error. Further effort toward improvement will be made during the summer if Dr. Craig can find the time.

C. Iodine number.

The method given by Yasuda (Jour. Biol. Chem. V 194, pp 401-409, 1931) is being used without modification. This is a recognized method and is proving satisfactory.

RESULTS OF TESTS

A. Conditioning exteriments.

Table I

PERCENT OF MORTALITY AT CRITICAL TEMPERATURES; AND FAT AND MOISTURE CONTENT.

Table I

Exposure	D. monticolae					D. brevicomis	
	Field*	18°	32°	36°	41-18°	Field	36°
Check	-	0	2	0	0	16	0
10	3	-	-	-	-	4	-
½	93	48	24	-	-	84	0
2½	96	48	24	-	-	84	-
0	100	98	40	19	2	96	24
-2½	-	-	41	-	10	-	-
-5	-	100	92	92	31	-	74
-7½	-	-	100	100	64	-	-
-10	-	-	100	-	98	-	90
-12½	-	-	-	-	-	-	100
Moisture %	-	61.0	59.6	60.4	59.2	-	51.3
Fat %	-	9.4	10.3	9.5	10.2	-	19.9
" Milligms per larva	-	1.94	2.13	2.0	2.53	-	2.80
Iodine Number	-	95.9	102.6	121.2	115.3	-	90.6
Saponification number	-	-	199.5	-	183.0	-	182.5

* Designations:

D. monticola

Field - as brought in from field Nov. 5. 1937.
18° - held at 18° for 32 days.
32° - held at 32° for 32 days.
36° - held at 36° for 32 days
41°-18° - held 10 days at 41°, 10 days at 32°, and 12 days at 18°.

D. brevicomis

Field - as brought in from field Nov. 10, 1937.
36° - held 35 days at 36°.

B. Seasonal tests - March 1938.

Exposures	D. monticolae - Central Sierra		D. brevicomis, Modoc.
	Host, sugar pine	Host lodgepole pine	
	Mortality %	Mortality %	Mortality %
Check	4	2	5
15°	6	-	-
12½°	-	-	-
10°	64	-	-
7½°	-	-	-
5°	70	-	-
2½°	78	-	-
0°	97	-	-
-2½°	-	14	77
-5°	-	44	100
-7½°	-	83	100
-10°	-	86	-
-12½°	-	98	-
-15°	-	98	-
Moisture %	32.0	24.6	54.2
Fat %	13.7	7.5	19.4
Fat - Milligms per larva	1.60	1.65	1.93
Iodine Number	102.0	107.0	83.4
Saponification number	177.8	171.0	169.0

COMMENTS

As stated in the first memorandum, the results of the conditioning experiments clearly demonstrate the "hardening" effect of sub-developmental temperatures. Of particular interest is the pronounced increase in resistance of the D. brevicomis larvae; this is the first time we have been able to artificially induce a change in this species.

In the seasonal tests the resistance was found to be approximately normal for this time of year except for the D. brevicomis larvae which were all killed at -5° instead of -7½° to -10° as is usual.

The results of the fat and moisture tests still leave much to be desired. In work with D. monticolae larvae there are no definite correlations although there are indications which may or may not be significant. It appears in the seasonal tests that the more resistant larvae (lodgepole pine) contained less water and as shown by the higher iodine number, had the lipids in a more reactive form. The same may have been true of the D. monticolae larvae in the conditioning experiments. However, in comparing the results from these two groups no such relationship exists, for the larvae from sugar pine has a much lower water content than any of the conditioned larvae, yet were less cold-resistant. The total fat content apparently had no relation to winter hardiness.

With D. brevicomis, the results are a little more encouraging although the data are too meagre for generalizations. Moisture and fat content were nearly the same in the two tests but there was a pronounced difference in the iodine and saponification values which would indicate that in the more resistant larvae the fatty acids are in shorter chains and contain more of highly reactive unsaturated bonds.

The most pronounced differences were to be found in comparing the lipids of the two species. Although D. brevicomis larvae contained a much higher percentage of fats than do D. monticolae larvae, the iodine and saponification values were both lower than in the fat from the latter species. Such a fat would be less available under low temperatures and so might be a factor in limiting the development and resistance of this less hardy species.

So far the results of our fat and moisture tests have shown little promise of supplementing freeze tests. I hope, however, that we may be able to continue this study through the next season; by that time we should have enough information to know whether or not this field shows any promise.

Respectfully submitted,

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Berkeley, California
March 31, 1938